

**REMARKS**

Reconsideration of the subject patent application is respectfully requested.

As the Examiner will note, a number of changes have been made to the specification in order to provide the suggested headings and to provide appropriate page breaks so that the various sections of the application each begin a new page. Additionally, the drawing descriptions have been moved to a point earlier in the application and, collectively, these amending changes are believed to put the entire application in the desired and appropriate form. With regard to the Abstract, the Examiner's comments are noted and a new Abstract has been provided. The amendments to the specification include deletion of the two Abstract paragraphs and the replacement of those two paragraphs by a new paragraph. This new paragraph does not exceed the 150 word limit.

With regard to the 35 U.S.C. §112 issues raised by the Examiner, a number of these were directed to claim 1 and that claim has been canceled. New claim 68 has been provided as a replacement for claim 1. It is believed that the various issues raised by the Examiner regarding claim 1 have been properly answered by the form and content set forth in new claim 68.

Additionally, a number of the claims have been canceled and other claims amended so as to establish a proper dependency and antecedent basis for the various dependent claims. Since all of the claims have either been amended or canceled, it may not be necessary to comment with regard to each of the issues raised by the Examiner with regard to the claims that are now canceled. Suffice it to say that Applicant did

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carefully consider the points raised by the Examiner and has tried very hard to address each of those by the various claim amendments, claim cancellations, and explanations that follow. Likewise, an effort has been made to place the application in the appropriate form. However, if any aspects in this regard have not been prepared to the satisfaction of the Examiner or if something has been inadvertently overlooked or omitted, Applicant would appreciate the Examiner pointing that out so that appropriate correction can be made.

With regard to the claim rejections and considering the new claims and the amended claims as set forth above, the Examiner is asked to consider the following remarks regarding the cited Zarling et al. patent. This is the only reference cited in the rejection of the claims, at least with regard to those claims still pending. It is noted that a rejection was set forth relative to claim 29 under 35 U.S.C. §103(a) based upon a combination of Zarling et al. and Selvin et al. However, since claim 29 has been canceled and the Selvin et al. patent was not relied upon for any other claim rejections, this particular prior reference does not need to be discussed.

The present invention relates to an energy transfer assay (see page 1 of the present specification) which is intended to overcome the disadvantages of prior energy transfer assays as summarised in the bullet points on page 3 of the present specification. The essence of the present invention is that an up-converting energy source is provided that is excited by long wavelength radiation (e.g. infra-red radiation) and that this energy source can transfer up-converted energy to an acceptor, for example by virtue of overlap of absorption spectrum of the absorbing species with the luminescence emission spectrum of the energy source. The transfer of energy is sensitive to proximity between the

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absorbing acceptor and the emissive donor and for a given spatial separation between donor and acceptor is often sensitive to the extent of spectral overlap between the emission of the energy donor and the absorption of the energy acceptor. The assay can be used to detect an analyte, for example by virtue of the ability of the analyte to change either the said spectral overlap (e.g. by changing the colour of the acceptor species) or to change the proximity between donor and acceptor species (e.g. by forming or dissociating a complex between them), or to change the luminescence properties of the acceptor species.

For a brief explanation of up-converting medium please see page 4, lines 16-19 of the present specification as well as the references in Zarling et al column 12, lines 61-65 and the specific examples shown in his Figs 5A-C as described at column 35, lines 13-40.

Zarling does disclose the use of up-conversion energy sources as labels in an assay but does not disclose an energy transfer assay, still less one as defined in amended claim 1 and summarised above. More particularly, for the purposes of an assay, Zarling uses the up-conversion energy media "simply" as a label to detect a binding event. In this respect, please see the disclosure in Zarling running from column 19, line 65 to column 21 line 5. A basic understanding of Zarling's use of up-converting media may be obtained from his Figs 20 and 23 and the related disclosures at column 49, lines 29-44 and column 54, line 44, column 55 line 2.

In summary, therefore, Zarling's assay "simply" seeks to detect the up-conversion medium itself as a label. In contrast, the present invention uses the up-conversion medium as a donor species for energy transfer to an acceptor species as defined in claim

1 and summarised above. The invention overcomes the disadvantages set out as "bullet points" on page 3 of the present specification.

On pages 15-18 of the Official Action the Examiner has provided detailed comments on (allegedly) relevant portions of the disclosure of Zarling et al. The following comments follow the Examiner's train of reasoning and address the points raised therein.

1) On page 15, last two lines and page 16, first two lines of the Official Action, the Examiner states that 'Additionally the solid phase may be provided with a continuous, discontinuous or partial coating of an (alkali) metal upon which polypeptide ligands and probes having up-converting labels are attached thereto (see column 13 and column 18, lines 48-60).'

This is at least in part a quote from the present application with the parenthetical addition of 'alkali'. We would however respectfully submit that the Examiner's comment appears to be based on a misunderstanding of the cited text of Zarling et al.

Zarling et al discuss coating of labels with Ludox, which is an aqueous colloidal silica suspension and is not metallic. The misunderstanding arises because the text mentions that the Ludox contains an 'alkali metal'. What this actually means is that Ludox contains alkali metal salts. As will be appreciated alkali metals cannot exist in aqueous media as they immediately react with water.

Thus Zarling et al make no reference to metal coating of up-converting particles as contemplated in the application.

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2) On page 16, lines 5-9 of the Official Action it is stated that 'The solid phase may also be in the form of paramagnetic particles which have a metal coating and which specifically have analyte (glycoprotein) binding molecules (immunoglobulins) capable of directly or indirectly binding acceptor species of the up-converting labels so as to bring them into close proximity with the metal (see column 25, lines 1-32).'

We can find no reference to such metal coating in the cited text. The examiner's comment appears to be based on a misunderstanding of the nature of the magnetic carrier and of the purpose for which the carrier is attached to the up-converting particle according to the claims of Zarling et al.

The magnetic material described by Zarling et al is in the form of superparamagnetic beads. There is no mention of metal particles and metal is not needed for superparamagnetism. In fact most superparamagnetic materials use colloidal magnetite (iron oxide) as the magnetic material. In superparamagnetic beads the bead material is typically a polymer mixed with colloidal magnetic material.

The reason for use of superparamagnetic beads coated with ligands is merely as a capture surface to bind up-converting particles (which simply act as detectable markers). The superparamagnetic beads can be collected by magnetically assisted separation, distinguished according to (e.g.) their size and tested for the presence of bound up-converting particles by measurement of the characteristic up-converted luminescence. There is no suggestion in Zarling et al that the proximity to the superparamagnetic beads influences the luminescence of the up-converting particles.

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The present application discloses metal coatings on solid phase (e.g. on an up-converting nanoparticle itself) and discusses the potential for such metal to accept energy from the particle. This is wholly different from the situation discussed by Zarling et al.

3) On page 16, lines 16 to 21 of the Official Action it is stated that 'Generally the up-converting lanthanide phosphor particles are an activator couple comprising ytterbium as the donor species or primary absorbing species of electromagnetic radiation and erbium or thulium as the acceptor species (emitter center) upon which the adsorbed energy from the donor species is ultimately transferred to excite the acceptor species. The acceptor species are ions of erbium or thulium.'

While this is an accurate description of the principle of the up-converting labels disclosed by Zarling et al it does not bear on the present claims in any way. The present application claims an assay based on energy transfer from the **up-converting** species to an energy acceptor bound in close proximity to the up-converting donor. The radiation used to excite the up-converting species of the present invention is not able to excite the acceptor directly by single photon absorption because the photon energy is too low. By contrast the process described by Zarling et al is a conventional energy transfer process from a donor to an excited state of an acceptor of equal or lesser energy. The energy transfer process described by Zarling et al is a sequential internal energy transfer from a **down-converting** sensitiser to an acceptor. The energy acceptor (e.g. erbium or thulium), not the energy donor (ytterbium), is the up-converting species.

The energy transfer process described is not a necessary part of the upconversion process and it is not necessary to include ytterbium as a sensitiser since erbium can be excited directly to give upconverted radiation. The ytterbium ions allow a single infrared

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wavelength to be used efficiently in the upconversion process, whereas direct excitation of erbium is optimally achieved with a combination of wavelengths.

4) On page 16, penultimate line to page 17, line 1 of the Official Action, it is stated that 'The transfer of energy from the donor to the acceptor is detected by decrease in luminescence efficiency of the donor species.' This is a quote from the present application given in the context of the internal energy transfer process from ytterbium to erbium ions which is discussed above.

Although detection of energy transfer by measurement of decrease in luminescence efficiency of an energy donor is a common procedure for conventional assays based on energy transfer between down-converting organic dyes we are unable to find any reference to such a procedure in the patent of Zarling et al. in the context of an up-converting energy donor as claimed in the present application.

5) On page 17, lines 5-9 of the Official Action, it is stated that 'In practice, Zarling et al teach irradiating the donor species with electromagnetic radiation to excite the donor species, then detecting luminescence in a defined spectral region of emission only by the donor species or the acceptor species wherein no absorption of radiation used to excite the acceptor species is measured.'

With respect, we find this sentence rather confusing as it is unclear what is intended by 'wherein no absorption of radiation used to excite the acceptor species is measured'. If, as we believe, the statement as a whole means that Zarling excites the internal energy donor (e.g. ytterbium ion) and measures luminescence from the emissive acceptor ion (e.g. erbium ion), then this merely defines the internal energy transfer process sometimes used to excite up-converting materials. As stated previously this is not

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transfer of up-converted luminescence to an energy acceptor, but is transfer of energy from a down-converter as is conventional in resonance energy transfer.

6) It is further stated (page 17, lines 15-17 of the Official Action) that 'The presence of analyte causes a change in the excitation condition of either one of the donor species or the acceptor species as a result of direct binding, indirect binding or release from the solid phase.'

This is a quote from the present application. However, Zarling et al make no such disclosure or claim anywhere in their patent specification. In Zarling's description analytes increase or decrease the binding of up-converting agents to surfaces facilitating the separation and measurement of the up-converting species by localising them. There is no suggestion in Zarling's disclosure that analyte binding of the up-converting species to the surface (as contemplated by Zarling) influences the spectroscopic properties of the up-converting species.

7) On page 17, line 18 to page 18, line 6 of the Official Action it is stated that 'Zarling et al also provide that the up-converting phosphors are used to magnetically or optically trap, i.e. electron-trapping phosphors, particles that comprise the analyte or the labeled probe'

The reference to 'optical trapping' is in no way relevant to the present application. 'Optical trapping' refers to the use of intense light beams to physically confine particles to a defined region and to move them spatially in response to changes in the incident illumination. The term 'optical tweezers' is often used to describe this process which is very well known.

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In the present application reference is made to 'electron trapping phosphors'. These are materials sometimes referred to as 'storage phosphors' where excitation of a semiconductor with visible or UV light or with ionising radiation generates hole-electron pairs where some of the excited electrons are 'trapped' in metastable states. Subsequent excitation of the metastable states with long wavelength radiation 'releases' the electrons, which can then undergo recombination processes leading to the emission of shorter wavelength radiation than the secondary stimulus.

Zarling et al do not consider the possible use of 'electron trapping phosphors' in their specification, and focus on labels that are excited by multiple absorption of lower energy photons. This conclusion can be verified from the section of their patent where they define what is meant by the terms used in their document. In column 10, lines 24-28 for example, it is stated that 'The labels of the invention are up-converting labels, which means that the chemical substituent absorbs at least two photons at an excitation frequency and subsequently emits electromagnetic energy at a frequency higher than the excitation frequency.' In an electron-trapping phosphor by contrast the initial excitation ('electron trapping') is by a high-energy photon or by exposure to ionising radiation, whereas the emission is stimulated by a single photon of low energy radiation.

All of the compositions disclosed by Zarling et al are of the 'two-photon' up-converting type and nowhere in the patent is there any evidence that the applicants were aware of the existence of electron trapping phosphors as potential up-converting agents.

The essential difference between an 'electron trapping phosphor' and an 'up-conversion phosphor' as discussed in the documentation relevant to the present application is straightforward. An 'up-conversion phosphor' as disclosed by Zarling et al

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can be excited by a sufficiently intense source of illumination to give emission of shorter wavelength than the excitation. There is no requirement to 'charge' the material by prior exposure to more energetic radiation, nor will the material become 'depleted' and show reduced efficiency over time under conditions normally encountered in use.

An 'electron-trapping' phosphor by contrast requires a 'priming' dose of energy to excite charge carriers therein to metastable trapped states. This 'priming' dose must be of greater or at least equal photon energy to that of the trapping state (and hence of the radiation ultimately detected). The stimulation of the higher energy emission by exposure to long wavelength radiation results from 'detrapping' as charge carriers are stimulated to escape from their potential wells and can then recombine with 'holes' giving more energetic emission than that of the stimulus.

8) On page 17, final three lines of the Official Action, it is stated that 'Up-conversion labels may also rely on excitation of organic molecule (sensitizing dye) to an excited state which relaxes to a metastable state or triplet state which transfers its energy to a dissolved molecular oxygen molecule to yield an excited singlet oxygen molecule.'

While this is true, the disclosure is not relevant to the present claims. More particularly, the present claims are limited to detection of an analyte on the basis of the analyte being able to influence energy transfer to an acceptor, for example by changing the colour of the acceptor so as to change the spectral overlap with the donor emission or by changing the proximity between donor and acceptor (e.g. by forming or dissociating a complex between them). This is quite different from the disclosure of Zarling et al.

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Zarling et al use bound dyes either as a means to control the emission wavelength of their labels so as to make them distinguishable from one another (for example for spectral multiplexing) or as photophysical catalysts specifically for purposes other than detection. For example Zarling describe dye-mediated generation of singlet oxygen for applications such as localised cytotoxicity (see for example column 27, lines 27-34). Nowhere in Zarling et al is it suggested that an analyte can be detected or quantified on the basis of the analyte being able to influence the nature or extent of energy transfer from the upconverting label to a bound species.

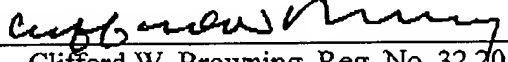
Singlet oxygen formation is a diffusion-controlled collisional process. There is no suggestion in Zarling et al that an analyte might influence the formation of singlet oxygen, nor is there any suggestion that the excitation condition of either the energy donor (dye or phosphor) or the acceptor (oxygen) might be monitored to determine an analyte.

Singlet oxygen formation can be detected chemically and this detection can form the basis of a proximity assay, but such detection is based on chemiluminescence of an oxidised species in the visible spectral region. For example, Zarling et al cite the use of dye sensitised chemiluminescence via oxidation of luminol by excited oxygen (column 4, line 67 to column 5, line 6). The interaction is a chemical oxidation reaction that consumes oxygen and is not an energy transfer process as claimed in our application. The excitation energy of singlet oxygen is approximately 1270nm which corresponds to an infra-red excitation and therefore singlet oxygen is insufficiently energetic to excite visible luminescence from an energy acceptor.

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In view of the amending changes and the remarks offered, claims 4-6, 8, 11, 17-20, 25, 46, 57-59 and 68-73 are believed to be in condition for allowance.

Respectfully submitted,

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